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Appl. No. 09/725,652

In the claims:

1. (Original) A method of detecting polynucleic acid polymerase activity, the method comprising:

(a) providing a polynucleic acid primer-template complex labeled with an energy-emitting chemical species and a nucleotide labeled with an energy-emitting chemical species;

(b) mixing the polynucleic acid primer-template complex and the nucleotide with a sample comprising or suspected to comprise a polynucleic acid polymerase;

(c) prior to, contemporaneously with or after the mixing of step (b), exposing the labeled polynucleic acid primer-template complex and the labeled nucleotide to radiation of excitation wavelength for one of the energy-emitting chemical species to thereby excite that energy-emitting chemical species; and

(d) detecting a signal produced by energy transfer between the excited energy-emitting chemical species and the other energy-emitting chemical species as a result of incorporation of the nucleotide into the polynucleic acid primer-template complex via the activity of the polynucleic acid polymerase, the detection of the signal indicating polynucleic acid polymerase activity in the sample.

2. (Original) The method of claim 1, wherein the nucleotide is selected from the group consisting of dUTP, dTTP, dATP, dCTP, dGTP, ATP, CTP, UTP, GTP and combinations thereof.

3. (Original) The method of claim 1, wherein the energy-emitting chemical species on the polynucleic acid primer-template complex is a donor chemical species and the energy-emitting chemical species on the nucleotide is an acceptor chemical species or wherein the energy-emitting chemical species on the nucleotide is a donor chemical species and the energy-emitting chemical species on the polynucleic acid primer-template complex is an acceptor chemical species.

4. (Original) The method of claim 1, wherein the energy-emitting chemical species on the polynucleic acid primer-template complex and the energy-emitting chemical species on the nucleotide are light-emitting chemical species.

5. (Original) The method of claim 4, wherein the light-emitting chemical species are each selected from the group consisting of a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, and a bioluminescent compound.

6. (Original) The method of claim 5, wherein the fluorescent compound is selected from the group consisting of fluorescein and derivatives thereof, rhodamine and derivatives thereof, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, fluorescamine, Texas red, cascade blue, Oregon green, phycoerythrin, CY3, CY5, CY2, CY7, coumarin, infrared 40, MR 200 and IRD 40.

7. (Original) The method of claim 5, wherein the light-emitting chemical species on the polynucleic acid primer-template complex, the light-emitting chemical species on the nucleotide or both the light-emitting chemical species on the polynucleic acid primer-template complex and the light-emitting chemical species on the nucleotide are rare earth metals.

8. (Original) The method of claim 7, wherein the rare earth metal light-emitting chemical species on the polynucleic acid primer-template complex, the rare earth metal light-emitting chemical species on the nucleotide or both the rare earth metal light-emitting chemical species on the polynucleic acid primer-template complex and the rare earth metal light-emitting chemical species on the nucleotide are lanthanides.

9. (Original) The method of claim 8, wherein the lanthanide further comprises a lanthanide chelate.

10. (Original) The method of claim 9, wherein the lanthanide chelate further comprises lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium or lutetium.

11. (Original) The method of claim 5, wherein the chemiluminescent compound is selected from the group consisting of luminol, isoluminol, theromatic acridinium ester and acridinium salt.

12. (Original) The method of claim 5, wherein the bioluminescent compound is selected from the group consisting of luciferin, luciferase and aequorin.

13. (Original) The method of claim 1, wherein the polynucleic acid polymerase is a DNA polymerase or a RNA polymerase.

14. (Original) The method of claim 13, wherein the polymerase is a reverse transcriptase.

15. (Original) The method of claim 1, further comprising detecting the signal at a plurality of time points over a predetermined time-period.

16. (Original) The method of claim 1, further comprising screening a plurality of samples simultaneously for polynucleic acid polymerase activity.

17. (Original) The method of claim 16, wherein steps (a) through (d) are carried out for each sample in a single well of a multi-well plate.

18. (Original) A method for identifying a candidate compound as a modulator of polynucleic acid polymerase activity, the method comprising:

(a) providing a candidate compound, a polynucleic acid primer-template complex labeled with an energy-emitting chemical species and a nucleotide labeled with an energy-emitting chemical species;

(b) mixing the candidate compound, the polynucleic acid primer-template complex and the nucleotide with a polynucleic acid polymerase;

(c) prior to, contemporaneously with or after the mixing of step (b), exposing the labeled polynucleic acid primer-template complex and the labeled nucleotide to

radiation of excitation wavelength for one of the energy-emitting chemical species to thereby excite that energy-emitting chemical species;

(d) detecting a signal produced by energy transfer between the excited energy-emitting chemical species and the other energy-emitting chemical species as a result of incorporation of the nucleotide into the polynucleic acid primer-template complex via the activity of the polynucleic acid polymerase, the detected signal indicating an amount of polynucleic acid polymerase activity; and

(e) identifying the candidate compound as a modulator of polynucleic acid polymerase activity based on the amount of signal detected as compared to a control sample.

19. (Original) The method of claim 18, wherein the nucleotide is selected from the group consisting of dUTP, dTTP, dATP, dCTP, dGTP, ATP, CTP, UTP, GTP and combinations thereof.

20. (Original) The method of claim 18, wherein the energy-emitting chemical species on the polynucleic acid primer-template complex is a donor chemical species and the energy-emitting chemical species on the nucleotide is an acceptor chemical species or wherein the energy-emitting chemical species on the nucleotide is a donor chemical species and the energy-emitting chemical species on the polynucleic acid primer-template complex is an acceptor chemical species.

21. (Original) The method of claim 18, wherein the energy-emitting chemical species on the polynucleic acid primer-template complex and the energy-emitting chemical species on the nucleotide are light-emitting chemical species.

22. (Original) The method of claim 21, wherein the light-emitting chemical species are each selected from the group consisting of a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, and a bioluminescent compound.

23. (Original) The method of claim 22, wherein the fluorescent compound is selected from the group consisting of fluorescein and derivatives thereof, rhodamine and derivatives

thereof, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, fluorescamine, Texas red, cascade blue, Oregon green, phycoerythrin, CY3, CY5, CY2, CY7, coumarin, infrared 40, MR 200 and IRD 40.

24. (Original) The method of claim 22, wherein the light-emitting chemical species on the polynucleic acid primer-template complex, the light-emitting chemical species on the nucleotide or both the light-emitting chemical species on the polynucleic acid primer-template complex and the light-emitting chemical species on the nucleotide are rare earth metals.

25. (Original) The method of claim 24, wherein the rare earth metal light-emitting chemical species on the polynucleic acid primer-template complex, the rare earth metal light-emitting chemical species on the nucleotide or both the rare earth metal light-emitting chemical species on the polynucleic acid primer-template complex and the rare earth metal light-emitting chemical species on the nucleotide are lanthanides.

26. (Original) The method of claim 25, wherein the lanthanide further comprises a lanthanide chelate.

27. (Original) The method of claim 26, wherein the lanthanide complex further comprises lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium or lutetium.

28. (Original) The method of claim 22, wherein the chemiluminescent compound is selected from the group consisting of luminol, isoluminol, theromatic acridinium ester and acridinium salt.

29. (Original) The method of claim 22, wherein the bioluminescent compound is selected from the group consisting of luciferin, luciferase and aequorin.

30. (Original) The method of claim 18, wherein the polynucleic acid polymerase is a DNA polymerase or a RNA polymerase.

31. (Original) The method of claim 30, wherein the polymerase is a reverse transcriptase.

32. (Original) The method of claim 18, further comprising detecting the signal at a plurality of time points over a predetermined time period.

33. (Original) The method of claim 32, further comprising calculating anassociation constant and a dissociation constant for the candidate compound for modulation of polynucleic acid polymerase activity.

34. (Original) The method of claim 32, further comprising calculating an IC_{50} value for the candidate compound for modulation of polynucleic acid polymerase activity.

35. (Original) The method of claim 18, further comprising screening a plurality of candidate compounds simultaneously for polynucleic acid polymerase modulator activity.

36. (Original) The method of claim 35, wherein steps (a) through (d) are carried out for each sample in a single well of a multi-well plate.

37. (Original) A method for identifying a candidate compound as a modulator of polynucleic acid polymerase activity, the method comprising:

(a) providing a candidate compound, a polynucleic acid primer-template complex labeled with a light-emitting chemical species and a nucleotide labeled with a light-emitting chemical species;

(b) mixing the candidate compound, the polynucleic acid primer-template complex and the nucleotide with a polynucleic acid polymerase;

(c) prior to, contemporaneously with or after the mixing of step (b), exposing the labeled polynucleic acid primer-template complex and the labeled nucleotide to radiation of excitation wavelength for one of the light-emitting chemical species to thereby excite that light-emitting chemical species;

(d) detecting a signal at a plurality of time points over a predetermined time period, the signal produced by energy transfer between the excited light-emitting

chemical species and the other light-emitting chemical species as a result of incorporation of the nucleotide into the polynucleic acid primer-template complex via the activity of the polynucleic acid polymerase, the detected signal indicating an amount of polynucleic acid polymerase activity; and

(e) identifying the candidate compound as a modulator of polynucleic acid polymerase activity based on the amount of signal detected as compared to a control sample.

38. (Original) The method of claim 37, wherein the nucleotide is selected from the group consisting of dUTP, dTTP, dATP, dCTP, dGTP, ATP, CTP, UTP, GTP and combinations thereof.

39. (Original) The method of claim 37, wherein the light-emitting chemical species on the polynucleic acid primer-template complex is a donor chemical species and the light-emitting chemical species on the nucleotide is an acceptor chemical species or wherein the light-emitting chemical species on the nucleotide is a donor chemical species and the light-emitting chemical species on the polynucleic acid primer-template complex is an acceptor chemical species.

40. (Original) The method of claim 37, wherein the light-emitting chemical species are each selected from the group consisting of a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, and a bioluminescent compound.

41. (Original) The method of claim 40, wherein the fluorescent compound is selected from the group consisting of fluorescein and derivatives thereof, rhodamine and derivatives thereof, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, fluorescamine, Texas red, cascade blue, Oregon green, phycoerythrin, CY3, CY5, CY2, CY7, coumarin, infrared 40, MR 200 and IRD 40.

42. (Original) The method of claim 40, wherein the light-emitting chemical species on the polynucleic acid primer-template complex, the light-emitting chemical species on the

nucleotide or both the light-emitting chemical species on the polynucleic acid primer-template complex and the light-emitting chemical species on the nucleotide are rare earth metals.

43. (Original) The method of claim 42, wherein the rare earth metal light-emitting chemical species on the polynucleic acid primer-template complex, the rare earth metal light-emitting chemical species on the nucleotide or both the rare earth metal light-emitting chemical species on the polynucleic acid primer-template complex and the rare earth metal light-emitting chemical species on the nucleotide are lanthanides.

44. (Original) The method of claim 43, wherein the lanthanide further comprises a lanthanide chelate.

45. (Original) The method of claim 44, wherein the lanthanide comprises lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium or lutetium.

46. (Original) The method of claim 40, wherein the chemiluminescent compound is selected from the group consisting of luminol, isoluminol, theromatic acridinium ester and acridinium salt.

47. (Original) The method of claim 40, wherein the bioluminescent compound is selected from the group consisting of luciferin, luciferase and aequorin.

48. (Original) The method of claim 40, wherein the light-emitting chemical species is a lanthanide chelate and the light-emitting chemical species is a fluorescent dye.

49. (Original) The method of claim 37, wherein the polynucleic acid polymerase is a DNA polymerase or a RNA polymerase.

50. (Original) The method of claim 49, wherein the polymerase is a reverse transcriptase.

51. (Original) The method of claim 37, further comprising calculating an association constant and a dissociation constant for the candidate compound for modulation of polynucleic acid polymerase activity.

52. (Original) The method of claim 37, further comprising calculating an IC_{50} value for the candidate compound for modulation of polynucleic acid polymerase activity.

53. (Original) The method of claim 37, further comprising screening a plurality of candidate compounds simultaneously for polynucleic acid polymerase modulator activity.

54. (Original) The method of claim 53, wherein steps (a) through (d) are carried out for each sample in a single well of a multi-well plate.